FINAL REPORT

CHARLOTTE HARBOR NATIONAL ESTUARY PROGRAM

Evaluating the risks that pharmaceutical-related pollutants pose to Caloosahatchee River wildlife: observations on the bull shark, *Carcharhinus leucas.*

Submitted to:

Judy Ott
Senior Scientist
Charlotte Harbor National Estuary Program
1926 Victoria Avenue
Fort Myers, FL 33901

Submitted by:

Dr. James Gelsleichter, Principal Investigator
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FL 34236

January 2009

Introduction

The problem that was addressed in this study is the growing concern about the presence of human pharmaceuticals in the aquatic environment (Daughton and Ternes, 1998). These chemicals include the parent compounds and metabolites of prescription and nonprescription drugs, such as contraceptives, antidepressants, anti-inflammatory drugs, and lipid-regulating agents. Pharmaceuticals are continually introduced into coastal ecosystems, primarily via excretion from human users and transport in domestic and industrial wastewater discharge. Despite this, a large majority of the most widely prescribed drugs in the United States have yet to be surveyed in the aquatic environment and research on the uptake and effects of these compounds in aquatic wildlife is in its infancy. Since many pharmaceuticals function by altering important biological processes that are common in most organisms, it is critical to determine the levels at which wildlife populations are exposed to these contaminants. This information is particularly vital for wildlife residing in coastal habitats bordering highly populated regions, such as Florida's southwest coast.

Given these concerns, the goal of this study was to characterize the potential health risks that pharmaceuticals pose to an aquatic wildlife species residing in the Caloosahatchee River, a wastewater-impacted tributary of the Charlotte Harbor estuary. To accomplish this goal, the presence and concentrations of active components of 8 widely prescribed drugs (Table 1) were measured in Caloosahatchee River surface water and blood of juvenile bull sharks (*Carcharhinus leucas*), an abundant and ecologically important resident of this river system (Heupel and Simpfendorfer, 2008). As an unplanned addendum to this study, we also examined the concentrations of human pharmaceuticals in wastewater effluent from a Ft. Myers sewage treatment plant that discharges treated wastewater directly to the Caloosahatchee River.

Materials and Methods

Environmental sampling

Environmental samples for measuring concentrations of pharmaceuticals in effluent and river water were obtained through the use of passive sampling devices known as Polar Organic Chemical Integrative Samplers (POCIS) (Alvarez et al., 2004). These devices contain a solvent-washed, solid-phase absorption medium that is capable of sequestering and concentrating a number of hydrophilic compounds including polar pesticides, prescription drugs, steroids, hormones, antibiotics, and personal care products. This resin is surrounded by two disc-shaped, semi-permeable polyethersulfone membranes that are held in place by two metal compression rings, which can be mounted on a specialized carrier. Previous studies conducted using these devices have demonstrated that this is a more efficient method for measuring waterborne polar contaminants than grab sampling, which provides data on only a single point in time. POCIS can be deployed for up to a month's time and provide data on the average concentrations of

environmental contaminants during this period. POCIS were deployed in duplicate at three sites in the tidal portion of the Caloosahatchee River between Cape Coral and Ft. Myers (Fig. 1) for a period of 30 days between mid-July and mid-August 2007 (n = 6). POCIS were also deployed in duplicate in the effluent and reclaimed water basins at the City of Ft. Myers Central Advanced Wastewater Treatment (AWWT) Facility (1501 Raleigh St., Ft. Myers, FL 33916) for a shorter duration (7 days) in October 2007 (n = 4). The Central AWWT Facility receives municipal sewage from Central and East Fort Myers as well as locations in Lee County as far away as Buckingham and Riverdale Shores. The facilitity discharges effluent directly to the Caloosahatchee River at a location just north of the Edison Bridge and adjacent to one of the surface water sampling sites (Site 2; Fig. 1).

Following their retrieval, POCIS were wrapped in acetone-rinsed aluminum foil and stored frozen at -20ºC until they were shipped on ice to Environmental Sampling Technologies, Inc. (St. Joseph, MO) for processing and extraction using proprietary methods. Briefly, extraction was conducted in chromatography columns using 40 mL of methanol per sampler. Extracts were concentrated to a volume of 1.5 mL using N_2 gas, filtered through glass fiber G-6 filter paper, and quantitatively transferred to 2-mL amber glass ampules using methanol. Samples were stored in vials until analyzed for concentrations of human pharmaceuticals.

Animal collection and biological sampling

Neonate bull sharks (60-95 cm in total length) were captured from regions in the Caloosahatchee River near POCIS deployment sites using longline fishing. A total of 40 sharks were collected between late May and early September of 2007, 12 of which were used to assess pharmaceutical exposure and uptake. The presence and concentrations of human drugs were also examined in 10 similarly-sized sharks collected from the same locations in 2006 and compared with 2007 data, in order to explore temporal changes in pharmaceutical exposure. In addition, 8 sharks were collected from locations in the lower Myakka River, a separate tributary of the Charlotte Harbor estuary that is not impacted by wastewater effluent, and were used as a reference group for this study.

Immediately following capture, blood samples (~5 mL) were obtained from sharks via caudal venipuncture using sterile syringes and 18-gauge needles, transferred to sterile tubes containing anti-coagulant, and stored on ice until returned to laboratory. Blood was centrifuged to separate plasma, which was stored in aliquots at -80ºC until shipped to the University of Florida's Analytical Toxicology Core Laboratory (ATCL) for measuring pharmaceutical concentrations. All sharks were tagged with plastic-tipped nylon dart tags and released live following blood sampling.

17α-ethynylestradiol analysis

Concentrations of the synthetic estrogen used in human contraceptives, 17α-ethynylestradiol (EE2), were measured in POCIS water extracts and shark plasma extracts using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Shark plasma was extracted twice with n-butyl chloride (4 mL per extraction) using a vortex mixer (60 s) and centrifuge (5 min at 1500 rpm). Measured volumes of POCIS extracts and the pooled organic layers from plasma extracts were concentrated to a residue under dry nitrogen, then dansylated via incubation (10 min at 60 ºC) in 0.1 mL of 0.1 M bicarbonate (pH 10.5) and 0.1 mL dansylchloride (2 mg/mL in acetone). The derivatized products were analyzed using a Hewlett-Packard HP1100 liquid chromatograph (Wilmington, DE) with tandem mass spectrometric detection (LCQ Ion Trap Mass Spectrometer; Finnigan MAT, San Jose, CA) in a method modified from Nelson et al. (2004). Analytes were introduced in a 50-µL injection and separated across an Adsorbosphere HS C18 column (250 mm x 4.6 mm x 5 µm; W.R. Grace & Co., Columbia, MD) under gradient conditions at a flow rate of 0.60 mL/min. Mobile phase A was 5:95 acetonitrile:water with 0.1% formic acid. The gradient began at 50% mobile phase B (95:5 acetonitrile:water with 0.1% formic acid), held for 2 min, increased to 95% B over 10 min, decreased back to 50% B over 5 min, and was allowed to equilibrate for 8 min. The retention time for EE2 was 24 min. Detection utilized MS/MS via APCI in positive ion mode. Although dansylation improved separation and ionization efficiency, greatest sensitivity was obtained by monitoring the unique ions belonging to EE2. The transition and collision energies monitored for EE2 and d_4-EE2 (a surrogate, as described below) were 530 to 448 at 34% and 534 to 470 at 36%, respectively.

Analytical grade standard for EE2 was purchased from Steraloids, Inc. (Newport, RI). Stock solutions were stored in PTFE-lined sealed screw cap bottles, with minimum headspace at a temperature between -10 and -20ºC and protected from light. Secondary dilution standards were prepared on a daily basis for the purposes of running calibration curves. The target analyte was quantified against a standard curve of at least six points having a correlation coefficient of at least 0.995. All standards and samples contained a surrogate (0.5 µg/mL of 17α-ethinylestradiol-d_4; CDN Isotopes Inc., Pointe-Claire, Quebec, Canada) that was fortified prior to derivatization, so that quantitation was against a ratio of analyte to internal standard response.

Other pharmaceutical analyses

Concentrations of the impotence agent sildenafil citrate, the lipid-lowering drug atorvastatin calcium, and the most commonly prescribed antidepressants in the United States, the selective serotonin reuptake inhibitors (SSRIs) sertraline, fluoxetine (and its primary metabolite, norfluoxetine), paroxetine, citalopram, fluvoxamine (in plasma of sharks collected in 2006 only) and the serotonin and norepinephrine reuptake inhibitor (SNRI) venlafaxine were measured in POCIS extracts and shark plasma using LC-MS/MS, following a method modified from those in

Frahnert et al. (2003) and Dallet et al (2002). Measured volumes of POCIS extracts (0.83 – 1.66 mL) were concentrated to a residue under dry nitrogen, then redissolved in 250 µL of mobile phase in preparation for analysis. Measured aliquots of shark plasma (1 mL) were centrifuged at 12,000 g for 10 minutes, after which supernatant was transferred to a new container, mixed with 2 mL of 0.1 M potassium dihydrogen phosphate butter (pH 6.0), and applied to an Empore mixed phase disk cartridge (3 mL; 3M Co., St Paul, MN) that was previously conditioned with 1 mL of methanol followed by 1 mL of water, each solvent being allowed to pass through the mixed phase via gravity. After sample loading, the cartridge was rinsed in order with 1 mL of water, 1 mL of 1 M acetic acid, 1 mL of hexane, 2 mL of 1:1 hexane: ethyl acetate, and 1 mL of methanol. The analytes were eluted in 2 mL of a 20:2:78 (v/v) 2-propanol: aqueous ammonia (25%): methylene chloride solution. Plasma extracts were concentrated to a residue under dry nitrogen and redissolved in 250 µL of mobile phase in preparation for analysis. Analytes were introduced in a 100-µL injection and separated across an Adsorbosphere HS C18 column (250 mm x 4.6 mm x 5 µm; W.R. Grace & Co., Columbia, MD) under isocratic conditions at a flow rate of 1.0 mL/min. The mobile phase consisted of a 40:60 (v/v) solution of acetonitrile: 10 mM aqueous ammonium acetate (pH 4.4). The retention times for venlafaxine, citalopram, sildenafil citrate, atorvastatin calcium, paroxetine, fluvoxamine, norfluoxetine, fluoxetine, sertraline, and bupivacaine (an internal standard, as described below) were 7.9 min, 12.9 min, 14.6 min, 16.4 min, 16.6 min, 19.5 min, 22.5 min, 28.3 min, 38.0 min, and 11.0 min, respectively. Detection utilized MS/MS via ESI in positive ion mode. The transitions and collision energies monitored for venlafaxine, citalopram, paroxetine, fluvoxamine, norfluoxetine, fluoxetine, sertraline, sildenafil citrate, atorvastatin calcium, and bupivacaine were 278 to 260 at 23%, 325 to 262 at 30%, 330 to 192 at 31%, 319 to 259 at 23%, 296 to 134 at 23%, 310 to 148 at 22%, 306 to 275 at 20%, 475.4 to 311 at 36%, 559.3 to 466 at 25%, and 289 to 140 at 30%, respectively.

Analytical grade standards were obtained from the following vendors: citalopram, paroxetine, fluvoxamine, norfluoxetine, fluoxetine, and sertraline from Cerilliant Corp. (Round Rock, TX), venlafaxine and bupivacaine from Sigma-Aldrich Co. (St. Louis, MO), sildenafil citrate from Pfeizer Inc. (New York, NY), and atorvastatin calcium from Toronto Research Chemical Inc. (North York, Ontario, Canada). Stock solutions were stored in PTFE-lined sealed screw cap bottles with minimum headspace at a temperature between -10º and -20º C and protected from light. Secondary dilution standards were prepared on a daily basis for the purposes of running calibration curves. All target analytes were quantified against curves containing at least six points ($R^2 \geq 0.995$). All standards and samples were fortified to contain an internal standard (0.1 µg/mL of bupivacaine), and analyte concentrations were quantified against a ratio of analyte to internal standard response. For plasma, duplicate quality control samples (nominally 100 ng/mL of venlafaxine, citalopram, paroxetine, fluvoxamine, norfluoxetine, fluoxetine, sertraline, and sildenafil citrate, percentage recovery ≥70% for each) were prepared

and analyzed alongside the actual samples. Due to the nature of the POCIS extracts, this type of quality control could not be performed.

Data analysis

As a reconnaissance of pharmaceutical concentrations in Ft. Myers effluent and Caloosahatchee River surface water (i.e., rather than a comparative study between multiple sites), data from POCIS extracts were presented in both raw format and as ranges of observed values for wastewater and river water separately for comparison with other studies. Pharmaceutical concentrations measured in POCIS extracts were adjusted by the total extract volume (1.5 mL) to present the total uptake of natural and synthetic estrogens in samplers in ng/POCIS, as presented in similar studies. The time-weighted average concentrations of EE2 in wastewater and river water were also determined from POCIS data using the equation (Alvarez et al., 2004):

$$C_w = \frac{C_s x M_s}{R_s x d}$$

where:

C_w = time-weighted average concentration of a given compound in water

C_s = the concentration of the compound in the POCIS sorbent

M_s = mass of the sorbent

R_s = the sampling rate of the compound (i.e., the volume of water cleared of analyte per unit of exposure time by the device).

A sampling rate of 0.2 L/d was used to calculate C_w of EE2 based on recent studies by Arditsoglou and Voutsa (2008), which reported average R_s values ranging from 0.2137 to 0.2217 L/d for this compound. As demonstrated by Zhang et al. (2008), field-derived sampling rates for estrogenic contaminants may be considerably (i.e., 2- to >12-fold) greater than those estimated from laboratory studies. Therefore, the use of 0.2 L/d as R_s was expected to provide us with a "worst-case" estimate of EE2 levels in Ft. Myers wastewater and Caloosahatchee River surface waters, as the use of higher sampling rates would yield lower estimates of EE2 concentrations. The range in time-weighted average concentrations of EE2 was presented for wastewater and river water separately for comparison with other studies. Time-weighted average concentrations were not determined for other pharmaceuticals because estimates of R_s are only available for a limited number of these compounds (i.e., fluoxetine, paroxetine).

Because only a limited number of shark plasma samples contained quantifiable levels of human pharmaceuticals, these data were grouped by site and year of collection and also presented as ranges of observed values. No statistical analyses were performed on these data.

Results

POCIS extracts

Quantifiable levels of 5 of the 8 human pharmaceuticals analyzed in the present study were detected in extracts from POCIS deployed in effluent and reclaimed water basins at the Ft. Myers Central AWWT Facility (Table 2). Of these compounds, the antidepressents venlafaxine and citalopram were most consistently detected in wastewater samples, typically at the greatest concentrations of all drugs surveyed. Sildenafil citrate and sertraline were also detected at comparable concentrations, but in extracts from only 1-2 of the 4 water samplers. EE2 was present in extracts from 3 of the 4 POCIS, but at the lowest concentrations of all drugs detected. Atorvastatin, paroxetine, and fluoxetine (and its primary metabolite, norfluoxetine) were not detected in any wastewater extracts.

Only 3 of the 5 pharmaceuticals found to be present in Ft. Myers wastewater were detected at quantifiable levels in extracts from at least 1 of the 4 POCIS recovered from the tidal Caloosahatchee River (Table 3). This included venlafaxine, citalopram, and EE2, all of which were found to be present at concentrations below those detected in wastewater effluent (i.e., on time-weighted basis). No other drugs were detected in surface water extracts.

Shark plasma

Detectable levels of 6 of the 8 human pharmaceuticals surveyed in this study were observed in the plasma of juvenile bull sharks collected from the Caloosahatchee River between 2006 and 2007 (Table 4). However, detection rates of these compounds in shark plasma varied considerably by year of capture. In 2006, all of the 6 antidepressants surveyed were detected in at least 1 of the 10 sharks examined; the most consistent of which was sertraline, which was found to be present in all animals. In contrast, only venlafaxine and citalopram were found to be present in plasma of Caloosahatchee River sharks sampled in 2007, both at low rates of detection. Similarly, while EE2 was not detected in plasma of Caloosahatchee River sharks sampled in 2006, it was found to be present in 7 of the 12 sharks examined in 2007. Despite these results, all pharmaceuticals that were detected in shark plasma were present in low concentrations, i.e., either below or slightly above limits of quantitation. Neither atorvastatin nor sildenafil citrate were observed in any Caloosahatchee River sharks. No pharmaceuticals were detected at quantifiable levels in plasma from Myakka River *C. leucas*.

Discussion

The results of this study indicate that human pharmaceuticals are present at detectable concentrations in incoming wastewater effluent and surface waters of the Caloosahatchee River. However, based on their low detection rate and concentrations in both river water and plasma of Caloosahatchee River bull sharks during the study period, these compounds do not currently

appear to pose significant health threats to bull shark populations residing in this river section. Nonetheless, as demonstrated by differences in the presence of pharmaceuticals in plasma of Myakka River and Caloosahatchee River bull sharks, the exposure and uptake of these compounds in bull sharks does appear to be influenced by the presence of wastewater treatment facilities in Charlotte Harbor tributaries. Furthermore, based on the temporal variations observed in pharmaceutical concentrations in plasma of Caloosahatchee River sharks, it is likely that changes in certain factors (e.g., prescription rates, population size, effluent discharge rates, precipitation, river flow, etc.) may influence the exposure of Charlotte Harbor bull sharks to these compounds. Therefore, it may be prudent to periodically monitor trends in environmental concentrations of certain highly prescribed pharmaceuticals, especially those that have been shown to cause effects in aquatic wildlife at relatively low levels of exposure (i.e., EE2).

Although it is often difficult to compare such studies due to differences in methodology and rapid advances in chemical detection capabilities, the EE2 concentrations observed in the present study were generally similar to those reported in prior surveys. For example, the estimated concentrations of EE2 in effluent from the Ft. Myers Advanced WWTP based on POCIS data (i.e., ND-2.30 ng/L) were comparable to earlier measurements of this compound in sewage from a number of industrialized countries including the U.S., Germany, Italy, Sweden, and the Netherlands (a range of ND-5.2 ng/L, Ying et al., 2002). The estimated concentrations of EE2 in Caloosahatchee River surface water (ND-0.23 ng/L) were also similar to levels detected in other U.S. water bodies, such as the 139 streams and creeks surveyed by Kolpin et al. (2002) (e.g., a range of ND-273 ng/L, but 94% of samples surveyed contained <5 ng/L EE2, Barnes et al., 2002). The overall consensus of this and other studies is that EE2 concentrations in U.S. waters are generally below the threshold levels necessary to induce biological effects. However, special concern regarding EE2 exposure in certain, highly impacted sites is warranted, as levels above the lowest observed effective concentration (LOEC) for inducing biochemical and organ-level effects in fish (1-10 ng/L and 10-100 ng/L, respectively; OECD, 2004) have been reported. For example, Kolpin et al. (2002) observed EE2 concentrations ranging from 31 to 273 ng/L in a small number of effluent-dominated U.S. water bodies. There is also reason for concern regarding the additive effects of multiple estrogenic pollutants in even moderately contaminated sites, as several studies have demonstrated that these compounds can act in a synergistic manner to induce biological effects in aquatic wildlife (e.g., Correia et al., 2007).

The lack of reliable estimates of POCIS sampling rates for other pharmaceuticals detected in Caloosahatchee River water and effluent from the Ft. Myers Advanced WWTP (i.e., sertraline, citalopram, venlafaxine, sildenfil citrate) precluded our ability to estimate ambient concentrations of these compounds in surface waters. This had little impact on our ability to compare the current findings with previous reports because little if any published data is available on the presence and concentrations of these compounds in U.S. waters. Nonetheless, it

is interesting to note that, like the present study, Schultz and Furlong (2008) recently reported that venlafaxine and citalopram were the most abundant SSRIs/SNRIs detected in a St. Paul, MN wastewater treatment plant and an effluent-dominated creek in Denton, TX. These findings appeared to be largely associated with the prevalent use of these drugs, as they have also been shown to be the most abundant SSRIs/SNRIs detected in St. Paul, MN sewage influent (Schultz and Furlong, 2008). This likely explains the present results as well, especially considering that citalopram and venlafaxine were the most prescribed SSRIs/SNRIs in the U.S. in 2007 (www.rxlist.com). Ironically, like the present study, sertraline was the third most abundant SSRI/SNRI detected in sewage effluent examined by Schultz and Furlong (2008). To the best of our knowledge, the present study is the first to report the detection of sildenafil citrate in environmental samples.

Although numerous studies have been conducted on the effects of EE2 in aquatic wildlife (e.g., Kidd et al., 2007), very little data are available on the uptake of this compound in fish for comparison with the present study. In fact, to the best of our knowledge, only two published papers have reported the detection of EE2 in wild fish (i.e., in bile) (Houtman et al., 2004; Gibson et al., 2005). However, the results of these studies could not be compared with the present findings because they were expressed in estrogen-equivalents, i.e., their ability to induce hormonal effects in estrogen-sensitive cell line bioassays. Nonetheless, the potential health risks that EE2 exposure currently pose to Caloosahatchee River bull sharks appears to be low, as all measurements of this compound in bull sharks were below or only slightly above the detection limit for this compound. Furthermore, as previously noted, the estimates of EE2 levels in river water derived from POCIS data fall well below the LOEC for health effects in aquatic species. It is also noteworthy to mention that, although preliminary, more recent work using common biomarkers of ecoestrogen exposure in fish (i.e., the estrogen-regulated yolk protein precursor, vitellogenin, Denslow et al., 1999) show no evidence of estrogenic effects in bull sharks sampled from this river system (Gelsleichter, unpublished data).

The concentrations of antidepressants detected in Caloosahatchee River bull sharks in both 2006 and 2007 were lower, but comparable with those observed in the only published study to investigate the uptake of these compounds in aquatic wildlife from U.S. waters. In this study, Brooks et al. (2005) reported concentrations of fluoxetine and sertraline ranging from 0.11-1.58 and 0.34-4.27 ng/mL, respectively, in various tissues (i.e., muscle, liver, and brain) of teleosts from an effluent-dominated Texas stream. A later study by Chu et al. (2007) also measured similar concentrations of fluoxetine (ND-1.02 ng/mL) and paroxetine (ND-0.58 ng/mL) in tissues of fish sampled from an urbanized embayment of Lake Ontario, Canada. As Brooks et al. (2005) concluded, these levels are generally low and unlikely to pose health risks to these species or individuals that consume them. This premise is also supported by experimental studies on the effects of fluoxetine on fish, which have shown that physiological alterations and mortality are

likely to occur only at exposure levels of SSRIs well above those reported in aquatic ecosystems (Brooks et al., 2004; Henry and Black, 2008). Nonetheless, it is premature to conclude that exposure to these compounds is entirely risk-free to Caloosahatchee River wildlife, particularly since only a limited number of studies on this topic have been conducted.

In summary, this study has demonstrated that commonly prescribed human pharmaceuticals are occasionally present at detectable concentrations in surface waters and bull sharks from the wastewater-impacted tidal Caloosahatchee River. However, since even the most prescribed group of drugs in the United States (i.e., antidepressants) is generally found in environmental levels below or just slightly above detection limits, it is unlikely that these compounds currently represent high priority risks to Charlotte Harbor bull sharks. Given these findings, greater emphasis may be placed on other water quality issues that threaten this river system, such as alterations in salinity associated with freshwater loads and/or nutrient enrichment. Nonetheless, future studies should continue to periodically monitor the presence of human pharmaceuticals in Caloosahatchee River and other wastewater-impacted sites in Charlotte Harbor, as the results from this study clearly demonstrate their potential for contaminating local water bodies. Additional work should also be conducted to evaluate the relationship between human activities (e.g., seasonal changes in population levels, prescription rates) and environmental factors (e.g., precipitation levels, water clarity and its effect on photolysis of pharmaceuticals) with pharmaceutical exposure and uptake in the diverse array of aquatic wildlife species that reside in the Charlotte Harbor estuary. Last, as championed in a recent review (Esplugas et al., 2007), much more work needs to be done to develop new technologies to improve the removal of pharmaceuticals and other micropollutants from wastewater effluents.

References

Alvarez DA, Petty JD, Huckins JN, Jones-Lepp TL, Getting DT, Goddard JP, and Manahan SE. (2004). Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ Toxicol Chem 23: 1640-1648.

Arditsoglou A, and Voutsa D. (2008). Passive sampling of selected endocrine disrupting compounds using polar organic chemical integrative samplers. Environ Pollut 156: 316-324.

Barnes KK, Kolpin DW, Meyer MT, Thurman EM, Furlong ET, Zaugg SD, and Barber LB. (2002). Water-quality data for pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000. U.S. Geological Survey Open-File Report 02-94.

Brooks BW, Chambliss CK, Stanley JK, Ramirez A, Banks KE, Johnson RD, and Lewis RJ. (2005). Determination of select antidepressants in fish from an effluent-dominated stream. Environ Toxicol Chem 24: 464-469.

Brooks BW, Foran CM, Richards SM, Weston J, Turner PK, Stanley JK, Soloman KR, Slattery M, and La Point TW. (2003). Aquatic ecotoxicology of fluoxetine. Toxicol Lett 142:169-183.

Chu S, and Metcalfe CD. (2007). Analysis of paroxetine, fluoxetine and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography-tandem mass spectrometry. J Chromatogr A 1163:112-118.

Correia AD, Freitas S, Scholze M, Goncalves JF, Booij P, Lamoree MH, Mananos E, and Reis-Henriques MA. (2007). Mixtures of estrogenic chemicals enhance vitellogenic response in sea bass. Environ Health Perspect 115 Suppl 1, 115-121.

Dallet P, Labat L, Richard M, Langlois MH, and Dubost JP. (2002). A reversed-phase HPLC method development for the separation of new antidepressants. J Liquid Chromatogr Rel Technol 25:101-111.

Daughton CG, and Ternes TA. (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 107 Suppl 6:907-938.

Denslow ND, Chow MC, Kroll KJ, and Green L. (1999). Vitellogenin as a biomarker of exposure for estrogen or estrogen mimics. Ecotoxicol 8: 385-398.

Esplugas S, Bila DM, Krause LGT, and Dezotti M. (2007). Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDC) and pharmaceuticals and personal care products (PPCPs) in water effluents. J Hazard Mater 149: 631-642.

Frahnert C, Rao ML, and Grasmäder K. (2003). Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. J Chromatogr B 794: 35-47.

Gibson R, Smith MD, Spary CJ, Tyler CR, and Hill EM. (2005). Mixtures of estrogenic contaminants in bile of fish exposed to wastewater treatment works effluents. Environ Sci Technol 39: 2461-2471.

Heupel MR, and Simpfendorfer CA. (2008). Movement and distribution of young bull sharks *Carcharhinus leucas* in a variable estuarine environment. Aquat Biol 1: 277-289.

Henry TB, and Black MC. (2008). Acute and chronic toxicity of fluoxetine (selective serotonin reuptake inhibitor) in western mosquitofish. Arch Environ Contam Toxicol 54: 325-330.

Houtman CJ, Van Oostveen AM, Brouwer A, Lamoree MH, and Legler J. (2004). Identification of estrogenic compounds in fish bile using bioassay-directed fractionation Environ Sci Technol 38: 6415-23.

Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW. 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc Natl Acad Sci USA 104: 8897-8901.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, and Buxton HT. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environ Sci Technol 36: 1202-1211.

Organization for Economic Co-operation and Development (OECD). (2004). Detailed review paper on fish screening assays for the detection of endocrine active substances. OECD Environment Directorate, Paris.

Schultz MM, and Furlong ET (2008). Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. Anal Chem 80:1756-1762.

Zhang Z, Hibberd A, and Zhou JL. (2008). Analysis of emerging contaminants in sewage effluent and river water: comparison between spot and passive sampling. Anal Chim Acta 607: 37-44.

Ying GG, Kookana RS, and Ru YJ. (2002). Occurrence and fate of hormone steroids in the environment. Environ Int 28: 545-551.

Table 1. Human pharmaceuticals measured in the present study. Limits of detection (LOD) and quantitation (LOQ) for each compound are presented for 0.5-mL POCIS extracts and 1-mL shark plasma samples separately. NM = not measured.

Chemical	Trade name	LOD-LOQ range	
		POCIS	Plasma (ng/mL)
Steroids			
17α-ethynylestradiol	Various (e.g., Ortho Evra)	0.02-0.50	0.10-1.00
Antidepressants			
Citalopram	Celexa Lexapro	3.00-6.25	0.10-0.25
Fluoxetine	Prozac Various generics	0.30-1.25	0.15-0.25
Norfluoxetine	Metabolite of fluoxetine	0.30-1.25	1.50-2.50
Fluvoxamine	Luvox	NM	0.15-0.25
Paroxetine	Paxil	0.30-1.25	0.50-1.25
Sertraline	Zoloft	0.80-2.50	0.10-0.25
Venlafaxine	Effexor	3.00-6.25	0.10-0.25
Impotence agents			
Sildenafil citrate	Viagra	0.30-1.25	3.00-6.25
Lipid-lowering drugs			
Atorvastatin calcium	Lipitor	0.05-0.25	0.30-1.25

Table 2. Presence and concentrations of human pharmaceuticals in duplicate POCIS deployed in effluent and reclaimed water basins at the City of Ft. Myers Central Advanced Wastewater Treatment Facility for a duration of 7 d. Values are in ng/POCIS. The time-weighted average (TWA) concentrations of EE2 were calculated as described in the text and provided as a range of values in ng/L. ND = not detected, NE = not estimated due to limited information on POCIS sampling rates for the chemical in question.

Chemical	Effluent			Reclaimed water		
	A	B	TWA	A	B	TWA
Steroids						
17α-ethynylestradiol	1.32	2.81	0.95-2.00	3.22	ND	ND-2.30
Antidepressants						
Citalopram	30.75	39.39	NE	77.97	82.76	NE
Fluoxetine	ND	ND		ND	ND	
Norfluoxetine	ND	ND		ND	ND	
Paroxetine	ND	ND		ND	ND	
Sertraline	ND	ND		49.04	61.89	NE
Venlafaxine	56.96	78.07	NE	150.90	167.30	NE
Impotence agents						
Sildenafil citrate	ND	ND		ND	17.43	NE
Lipid-lowering drugs						
Atorvastatin calcium	ND	ND		ND	ND	

Table 3. Concentrations of human pharmaceuticals in duplicate POCIS deployed at two sites in the tidal Caloosahatchee River for a duration of 30 d. A third set of POCIS deployed at Site 2 was lost in the field. Values are in ng/POCIS. The time-weighted average (TWA) concentrations of EE2 was calculated as described in the text and provided as a range of values in ng/L. ND = not detected, NE = not estimated due to limited information on POCIS sampling rates for the chemical in question. *The concentrations of citalopram and venlafaxine presented for Site 4 were determined using pooled extracts from POCIS A and B because of an injection error that occurred during SSRI/SNRI analysis, which resulted in partial loss of the individual samples.

Chemical	Site 4			Site 9		
	A	B	TWA	A	B	TWA
Steroids						
17α-ethynylestradiol	ND	ND		1.25	1.40	0.21-0.23
Antidepressants						
Citalopram	*138.82		NE	32.65	ND	NE
Fluoxetine	ND	ND		ND	ND	
Norfluoxetine	ND	ND		ND	ND	
Paroxetine	ND	ND		ND	ND	
Sertraline	ND	ND		ND	ND	
Venlafaxine	*122.91		NE	ND	ND	
Impotence agents						
Sildenafil citrate	ND	ND		ND	ND	
Lipid-lowering drugs	ND	ND		ND	ND	
Atorvastatin calcium	ND	ND		ND	ND	

Table 4. Concentrations of human pharmaceuticals in plasma of juvenile bull sharks (n = 30) collected from the Caloosahatchee (2006, 2007) and Myakka (2007 only) Rivers. Due to the limited number of quantifiable observations, values are presented as ranges of concentrations in ng/mL. The number of detectable observations below (BQL) and above (>LOQ) the limit of quantitation are shown in parentheses. ND = not detected. NM = not measured.

Chemical	Myakka River	Caloosahatchee River	
	(n = 8)	2006 (n = 10)	2007 (n = 12)
Steroids			
17α-ethynylestradiol	ND	ND	ND-3.79 (7 BQL, 2 >LOQ)
Antidepressants			
Citalopram	ND	0.25-0.57 (5 >LOQ)	ND-BQL (1 BQL)
Fluoxetine	ND	ND-BQL (1 BQL)	ND
Norfluoxetine	ND	ND-4.08 (1 >LOQ)	ND
Fluvoxamine	NM	ND-0.90 (4 >LOQ)	NM
Paroxetine	ND	ND-0.55 (1 >LOQ)	ND
Sertraline	ND	BQL-0.97 (1 BQL, 9 >LOQ)	ND
Venlafaxine	ND-BQL (1 BQL)	ND-0.32 (3 >LOQ)	ND-0.56 (2 >LOQ)
Impotence agents			
Sildenafil citrate	ND	NM	ND
Lipid-lowering drugs			
Atorvastatin calcium	ND	NM	ND

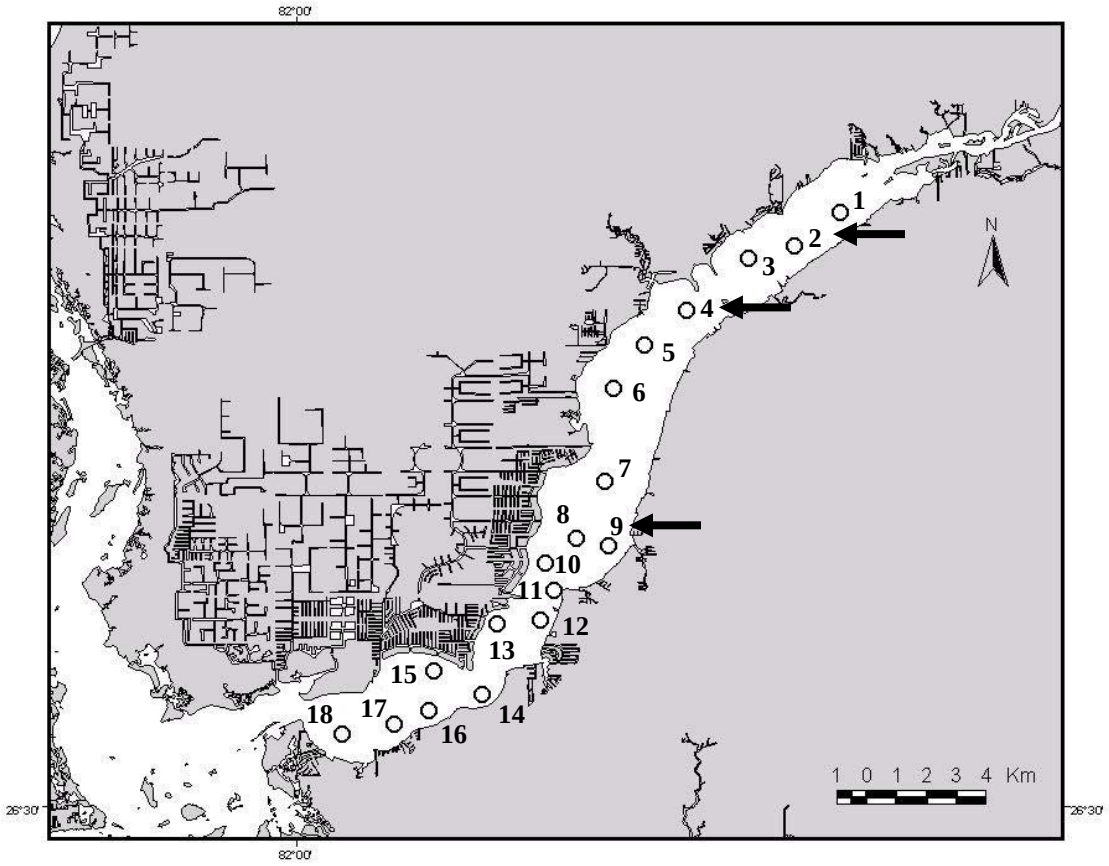

Figure 1. Location of acoustic receiver monitoring sites in the tidal Caloosahatchee River used to monitor the movements of juvenile bull sharks in previous studies (Heupel and Simpfendorfer, 2008). Arrows demonstrate the numbered sites (2, 4, and 9) where POCIS deployed in the present study. . The POCIS deployed at Site 2 was not recovered. Sub-surface discharge of wastewater effluent from the Ft. Myers Central Advanced Wastewater Treatment Facility occurs near Site 2. Sharks were collected between sites 1 and 7.